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Evolution of Developmental Control Mechanisms

Hierarchical evolution of animal body plans

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ABSTRACT

An open question in animal evolution is why the phylum- and superphylum-level body plans have changed so little, while the class- and family-level body plans have changed so greatly since the early Cambrian. Davidson and Erwin (Davidson and Erwin, 2006; Erwin and Davidson, 2009) proposed that the hierarchical structure of gene regulatory networks leads to different observed evolutionary rates for terminal properties of the body plan versus major aspects of body plan morphology. Here, we calculated the speed of evolution of genes in these gene regulatory networks. We found that the genes which determine the phylum and superphylum characters evolve slowly, while those genes which determine the classes, families, and speciation evolve more rapidly. This result furnishes genetic support to the hypothesis that the hierarchical structure of developmental regulatory networks provides an organizing structure which guides the evolution of aspects of the body plan.

Introduction

The Cambrian explosion has been an extensively debated topic in animal evolution for more than one century (Darwin, 1859; Walcott, 1914; Wray et al., 1996). Biological organisms were composed of individual cells, occasionally organized into colonies, before the Cambrian explosion (Wray et al., 1996; Hedges et al., 2004). Subsequent to the Cambrian explosion, evolution greatly sped up, and the major phyla appeared. For example, the bilateral, anterior–posterior organization of body plan appears in fossil records from the early Cambrian (Chen, 2004). These results are the basis for the open question in animal evolution of why the phylum- and superphylum-level body plans have changed so little and no more new phylum- and superphylum-level body plans appeared, while the class- and family-level body plans have changed so greatly with so many class, family, and species appearing since the early Cambrian (Valentine, 2004). Since the development of the animal body plan is precisely controlled by gene regulatory networks, the mechanism to explain the different rates of change of the phylum- and superphylum-level body plans versus the class- and family-level body plans may lie in the structure and evolution of gene regulatory networks.

If the gene regulatory network were an unstructured or nearly random network, any change to the network such as deleting one gene would result in drastic difference in the body plan because each gene may regulate or be regulated by several other genes, and the effects of deletion will spread out to the whole network quickly (Barabási and Zoltán, 2004). To resolve this problem, Davidson and Erwin (2006,2009) proposed that the classic evolution theory based on selection of changes upon an unstructured genetic framework does not provide a satisfactory answer for the mechanism. Instead, they constructed the gene regulatory networks that control the early development of animal embryos (see Fig. 1) (Levine and Davidson, 2005) and proposed a hierarchical modular structure of the gene regulatory network. The regulatory network of sea urchin endomesoderm specification genes expressed between 0 and 30 hours is composed of about 60 genes. This network is relatively modular. For example, as measured by the commonly used Newman modularity measure (Newman, 2006), defined as the fraction of edges that lie within modules rather than between modules relative to that expected by chance, the modularity of this gene regulatory network is 0.49. This modularity value greater than zero indicates that this network is quite modular.

Davidson and Erwin found that the gene regulatory network can be described by a hierarchy with four types of modules. The first type is named "kernel." For example, the endoderm specification kernel is composed of five genes in sea urchins, see Fig. 1. The heart-field specification kernel (Satou and Satoh, 2006; Cripps and Olson, 2002) is used in both *Drosophila* and vertebrate development. The other three types are named as "plug-ins," "I/O switches," and "batteries." Each type of module functions differently in the development of embryo. The kernels might relate to the phylum- and superphylum-level characteristics; the plug-ins and I/Os might relate to the class, order, and family characteristics; and the batteries might relate to the speciation characteristics (see Fig. 2). This proposal stimulated debate (Coyne, 2006; Erwin and Davidson, 2006). For example, the diverse kinds of changes in the hierarchy of gene regulatory networks and their evolutionary consequences are thought to be imperfect, and yet

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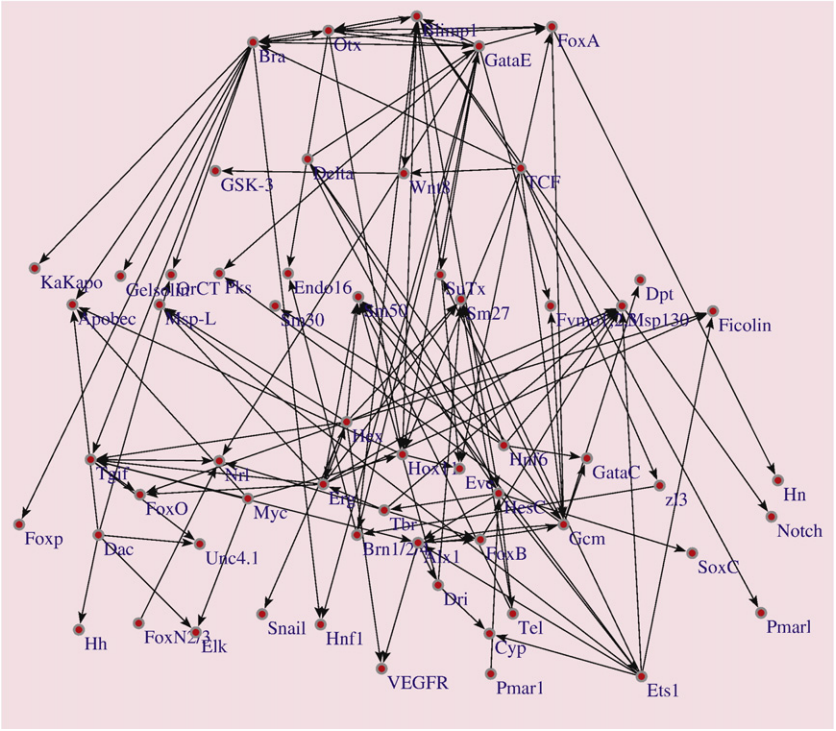

Fig. 1. The gene regulatory network of sea urchin endomesoderm specification up to 30 hours. The top five genes form the kernel. Data from http://sugp.caltech.edu/endomes/.

all essential major phylum-level body plans appeared at the early Cambrian. Davidson and Erwin stated that, "Critically, these kernels would have formed through the same processes of evolution as affect the other components, but once formed and operating to specify particular body parts, they would have become refractory to subsequent change."

If this theory is correct, we would expect the evolution of the gene regulatory network to be heterogeneous. The "kernels" module should evolve more slowly than other parts of the gene regulatory network, since the phylum- and superphylum-level body plan characteristics have not changed substantially since the early Cambrian. The gene regulatory networks are primarily composed of two elements: transcription factors and *cis*-regulatory modules. Transcription factors are proteins that can either activate or repress transcription by binding to *cis*-regulatory elements. Transcription factor binding sites are often organized into clusters named *cis*-regulatory modules, which typically span a few hundred nucleotides and can contain dozens of binding sites for several transcription factors (Chen and Rajewsky, 2007). A full understanding of the evolution of the gene regulatory network would consider both transcription factors and *cis*-regulatory modules. *cis*-Regulatory modules are poorly conserved during evolution, and even in closely related species may differ drastically (Wray, 2007; Chen and Rajewsky, 2007). Because experimental identification of *cis*-regulatory elements is still not well developed, and because computational prediction of *cis*-regulatory elements is still difficult (Elnitski et al., 2006), we considered only evolution of the transcription factors. Transcription factors are more conserved and evolve more slowly than *cis*-regulatory elements. On the timescale of hundreds of millions of years that we consider here, it is important to consider the evolution of transcription factor networks. For example, acquisition of an extra repressive regulatory domain in the insect protein *Ubx*

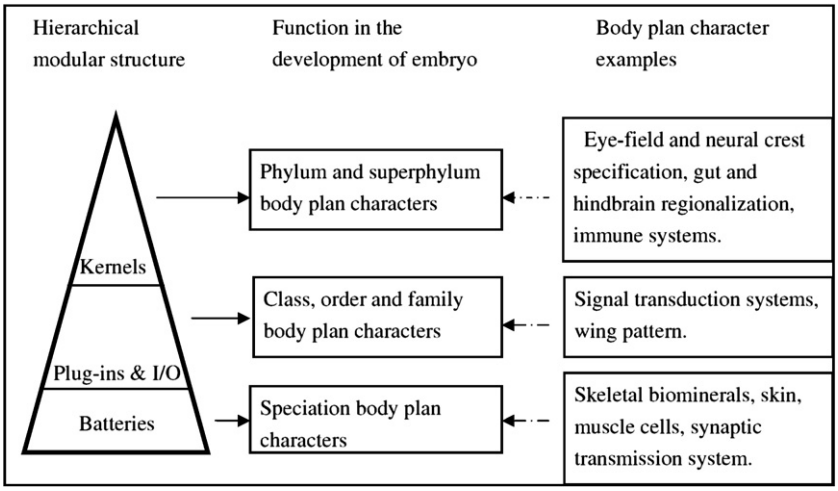

Fig. 2. The hierarchy of the gene regulatory network and functions at different levels of development of the body plan.

results in the prevention of the development of abdominal legs (Ronshaugen et al., 2002). Although transcription factors change slowly, their effect on the development body plan is of equal importance to that of *cis*-regulatory elements, since the variation of transcription factors directly changes the topology of the gene regulatory network. To test the theory of Davidson and Erwin, we calculated the speed of evolution of regulatory genes. We found that those genes which determine the phylum and superphylum characters evolve slowly, while those genes which determine the classes, families, and speciation evolve more rapidly. These observations provide support for Davidson and Erwin's theory.

Materials and methods

Sea urchin gene regulatory network

The sea urchin is a traditional model organism in developmental biology. The sea urchin (*Strongylocentrotus purpuratus*) and sea star (*Asterina miniata*) are in the same phylum Echinodermata. The sea urchin is in the class Echinoidea, and the sea star is in the class Asteroidea. The last common ancestor of echinoid and asteroid existed about 0.5 billion years ago in the late Cambrian (Smith, 1988; Bowring and Erwin, 1998; Veronica et al., 2003). Other sea urchins we studied are *Hemicentrotus pulcherrimus*, *Paracentrotus lividus*, *Heliocidaris erythrogramma*, *Heliocidaris tuberculata*, and *Lytechinus variegatus*. They are in the same superorder Echinacea with *S. purpuratus*. The genome of the sea urchin *S. purpuratus* was sequenced in 2006. The gene sequences used in this paper were all downloaded from the EMBL-EBI database in July 2008. The experimentally determined sea urchin gene regulatory network was downloaded from http://sugp.caltech.edu/endomes/.

Ratio of nonsynonymous substitution to synonymous substitution of genes

We used the widely accepted ratio of the rate of nonsynonymous substitutions to the rate of synonymous substitutions (d*N*/d*S*) as a measure of the rate of evolution. Generally, d*S* is a measure of evolutionary divergence between two genes due to neutral substitution, and the d*N*/d*S* is the departure from the neutral substitution caused by functional constraints and selection. The larger the d*N*/d*S* value, the faster is the gene evolving due to selection. We used a standard method to calculate d*N*/d*S* (Hirsh et al., 2005). First, we obtain the genes in the gene regulatory networks of sea urchin endomesoderm, see Fig. 1. We applied Wu-BLAST2 to search the orthologous genes in the 7 genomes mentioned above from EMBL-EBI. We required all the protein pairs to be reciprocal best hits. Since these organisms are closely related, all orthologous proteins have the same name and likely perform similar functions. Then, we aligned the orthologous protein pairs in ClustalW (Thompson et al., 1994) and calculated the d*N*/d*S* in PAL2NAL (Suyama et al., 2006). For each gene, we averaged the d*N*/d*S* of all the protein pairs of that gene. Taking gene *Bra* as an example, we found *Bra* genes in sea urchins *H. pulcherrimus*, *P. lividus*, *L. variegatus*, and *S. purpuratus*. *Bra* should also appear in other sea urchins not yet sequenced. We align the *Bra* protein sequences in each two sea urchins, 6 pairs in total. For each pair, use PAL2NAL to calculate d*N*/d*S*, see Table 1.

Results

The d*N*/d*S* for genes in gene regulatory networks are listed in Table 1. The gene regulatory network is composed of transcription factor (TF) and non-TF proteins. Most TF genes are utilized for diverse interactions, and the DNA binding domains of all of them are highly conserved across Bilateria. We average the d*N*/d*S* of all proteins in each hierarchical level, and the result is shown in Fig. 3. The value of

Table 1
Evolutionary rate of regulatory genes in pairs of organisms (Org. 1 and Org. 2).

Group	Gene	d*N*/d*S*	Org. 1	Org. 2	d*S*	d*N*
Kernels	*FOXA*	0.0083	PATVU	STRPU	52.38	0.4328
Kernels	*KROX*	0.0114	ASTM	STRPU	57.1692	0.6491
Kernels	*OTX*	0.0703	ASTM	STRPU	5.1028	0.3587
Kernels	*OTX*	0.11	HEMPU	STRPU	0.109	0.012
Kernels	*OTX*	0.122	HEMPU	ASTM	4.2	0.51
Kernels	*GATAE*	0.015	ASTM	STRPU	31.6839	0.4776
Kernels	*BRA*	0.0435	HEMPU	PARLI	0.87	0.03
Kernels	*BRA*	0.0408	HEMPU	LYTVA	0.997	0.407
Kernels	*BRA*	0.057	PARLI	LYTVA	0.897	0.051
Kernels	*BRA*	0.0413	HEMPU	STRPU	0.19	0.0079
Kernels	*BRA*	0.0456	PARLI	STRPU	0.85	0.038
Kernels	*BRA*	0.0493	LYTVA	STRPU	0.82	0.04
Plug-ins	*WNT8*	0.147	HELER	STRPU	0.7104	0.1051
Plug-ins	*TCF*	0.07	PARLI	STRPU	0.59	0.04
Plug-ins	*TCF*	0.0324	HELER	STRPU	0.4	0.013
Plug-ins	*TCF*	0.0224	PARLI	HELER	0.429	0.009
Plug-ins	*DELTA*	0.15	STRPU	PARLI	0.45	0.066
Plug-ins	*DELTA*	0.14	PARLI	LYTVA	0.64	0.09
Plug-ins	*DELTA*	0.19	STRPU	LYTVA	0.47	0.09
Plug-ins	*GSK-3*	0.0557	PARLI	LYTVA	0.4002	0.0223
I/Os	*SM30*	0.053	STRPU	HEMPU	0.106	0.005
I/Os	*MSP130*	0.226	HELTB	HELER	0.218	0.049
I/Os	*MSP130*	0.174	HELER	STRPU	0.741	0.129
I/Os	*MSP130*	0.167	HELTB	STRPU	0.736	0.123
I/Os	*SM50*	0.2436	HEMPU	STRPU	0.1891	0.046
I/Os	*CAPK*	0.101	STRPU	HEMPU	0.0637	0.0064
Batteries	*HNF6*	0.0637	ASTM	STRPU	4.5405	0.2894
Batteries	*GSC*	0.129	STRPU	HELTB	0.59	0.076
Batteries	*GSC*	0.08	LYTVA	HELER	1.12	0.1
Batteries	*GSC*	0.09	STRPU	LYTVA	0.903	0.082
Batteries	*ALX1*	0.028	STRPU	LYTVA	0.79	0.02
Batteries	*ALX1*	0.048	LYTVA	PARLI	1.15	0.056
Batteries	*ALX1*	0.068	STRPU	PARLI	0.85	0.058
Batteries	*ETS*	0.072	STRPU	PARLI	0.866	0.062
Batteries	*KRL*	0.601	STRPU	HEMPU	0.128	0.0776
Batteries	*SOXB1*	0.055	STRPU	HELER	0.45	0.02
Batteries	*SOXB1*	0.0527	HELER	HELTB	0.078	0.004
Batteries	*SOXB1*	0.0568	STRPU	HELTB	0.4423	0.251
Batteries	*GCM*	0.0911	LYTVA	PARLI	2.61	0.23
Batteries	*GCM*	0.0951	PARLI	STRPU	0.963	0.0916
Batteries	*GCM*	0.1952	LYTVA	STRPU	1.1161	0.2179
Batteries	*HOX11*	0.1513	HELTB	HELER	0.0639	0.009
Batteries	*HOX11*	0.1082	HELER	STRPU	0.35	0.038
Batteries	*HOX11*	0.0888	HELTB	STRPU	0.386	0.0343

Group 1 are kernels, group 2 are plug-ins, group 3 are I/O, group 4 are batteries.
STRPU: *S. purpuratus*, PATVU: *Patella vulgata*, ASTM: *A. miniata*, HEMPU: *H. pulcherrimus*, PARLI: *P. lividus*, HELER: *H. erythrogramma*, HELTB: *H. tuberculata*, LYTVA: *L. variegatus*.

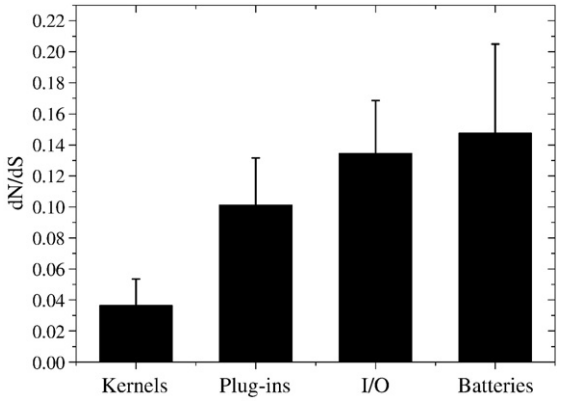

Fig. 3. The ratio of the rate of nonsynonymous substitutions to the rate of synonymous substitutions for different components of the gene regulatory networks that control the development of animal embryos. Standard errors are shown in figure. The kernels are at the top of the hierarchy, and the batteries are at the bottom of the hierarchy.

Table 2
P value of Wilcoxon test for different hierarchical levels.

Hierarchy	Hierarchy	*P* value
Kernels	Plug-ins	0.0826
Kernels	I/Os	0.0032
Kernels	Batteries	0.0092
Kernels	Plug-ins + I/Os + Batteries	0.0026
Kernels + Plug-ins	I/Os + Batteries	0.0157

The hypothesis for the Wilcoxon test is that two independent samples come from distributions with the same median.

dN/dS of kernels is significantly lower than plug-ins, I/Os and batteries, see Table 2 for *P* value. Also, we observe that the regulatory gene group of kernels and plug-ins has a lower dN/dS value than group of I/Os and batteries (relative difference = −0.055, *P* value = 0.0157 for Wilcoxon test). From the probability distribution of dN/dS in Fig. 4, we can see the distribution of kernels is narrow width, and the peak probability appears at a low dN/dS. If only TF genes are considered, kernels ($dN/dS = 0.045$) still evolve more slowly than other components. We also see slight increase of dN/dS from plug-ins to I/Os and to batteries ($dN/dS = 0.138$). Interestingly, we found that the number of organisms for which an ortholog was detected varies from genes to genes. For example, *A. miniata* is the least close organism to *S. purpuratus* compared to other sea urchins, *S. purpuratus* and *A. miniata* are in the same phylum but different orders. We found four orthologous genes between *A. miniata* and *S. purpuratus*. Three of them are kernel genes. The orthologs of kernel genes are more likely detected than other genes in far related organism, which is a support of slower evolution of kernels. Our results show that if two organisms are in the same phylum, their kernel modules that determine the phylum-level body plan are conserved. If two organisms are in the same class or order, their plug-ins and I/O modules are conserved since they determine the class and order level body plan.

Another supporting evidence comes from the "generative entrenchment" theory by William Wimsatt (Wimsatt, 1986; Wimsatt and Schank, 2004). In this model, the phenotype is considered as a generative structure. The generative structure of the system has a characteristic set of causal interactions which can be represented by the directed graph, see Fig. 1. In this model, nodes with more downstream connections should have slower evolution rates, since changes to them affect many epistatic interactions that must be accommodated during the evolution. Quantitatively, we account the downstream connections for each gene in gene regulatory networks. For example, if gene A regulates the expression of gene B, we say gene A has a downstream connection. We observe that kernels genes in Fig. 1 have an average of 5.4 downstream connections, while the other regulatory genes have an average of 1.6 downstream connections. All else being equal, nodes with more downstream connections such as kernels here should be more conservative, because their activity can bring more consequences. If they are changed, it is more likely that something will go wrong. One model to explain this idea was proposed by Riedl (1978). In this model, Riedl raised the idea of "burden" which states that the evolvability of a character change during evolution depends on the importance of the functions and structures depending on it. The kernels of the gene regulatory networks which determine the phylo-level body plan are thought to have "heavier" burden than other parts of gene regulatory works, since it is the base of animal body plan. So kernels are likely to be more conserved.

As additional supporting evidence, we consider the time of appearance of regulatory genes during embryo development. From available experimental data, we show the earliest appearance time of regulatory genes, defined as the time when a given gene is expressed and starts to regulate the expression of other genes. In Fig. 5, we can see that the kernel genes generally express earlier in endomesoderm than other regulatory genes, and most plug-ins genes appear earlier than I/Os genes. The Karl Ernst von Baer's law states that "General characteristics of the group to which an embryo belongs develop before special characteristics. General structural relations are likewise formed before the most specific appear." That is: differentiation proceeds from the general to particular, with taxonomically more general parts expressed earlier in development. In this case, we can interpret as the kernels which expressed earliest in development are more related to the higher hierarchical level of taxon such as phylum- and superphylum-level body plan, while others are more likely related to lower hierarchical level body plan. Genes which are expressed earlier in development are, mostly likely, older and more likely to be conserved during evolution, because mutations of proteins expressed earlier in embryo development are more likely to have larger, more pervasive, and more deleterious effects on subsequent development (Wimsatt and Schank, 2004).

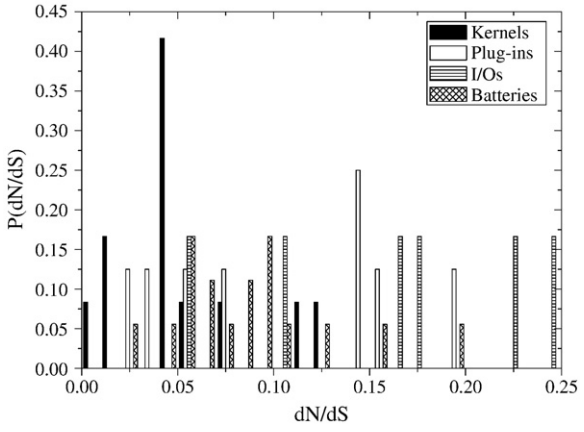

Fig. 4. The distribution of dN/dS for each hierarchical level. $P(dN/dS)$ is the probability of a gene with the dN/dS in specific hierarchical level.

Discussion

Recently, it has been found that biological networks are not random and unstructured networks; instead, many are modular networks (Newman, 2006). Modular network can be decomposed into several highly interacting modules and are particularly interesting. Perturbations or errors in a modular network are typically restricted to one module, and the effect on the whole network is

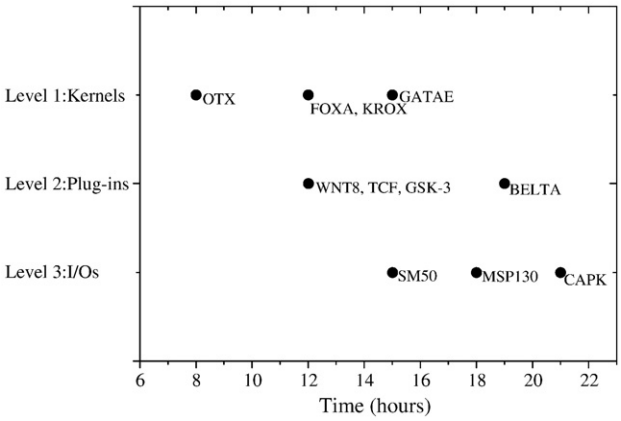

Fig. 5. The earliest appearance time of regulatory genes for kernels, plug-ins, and I/Os from experiment (Davidson et al., 2002). The figure shows the endomesoderm specification up to 22 hours for genes listed in Table 1. The *x*-axis is the appearance time of genes in embryo development, and the *y*-axis indicates in what hierarchical level genes belong to.

limited. Modular networks can evolve by rewiring the modules. This rewiring capability property tends to make modular networks more evolvable (Hartwell et al., 1999; Sun and Deem, 2007). A hierarchical network is an advanced modular network. In hierarchical networks, some modules are key modules that may relate to the core function and be resistant to mutation. Other modules are periphery modules that may be more likely affected by the environmental changes (Ravasz et al., 2002). Peripheral modules evolve rapidly and allow the organism to survive in a changing environment.

The origin of animal body plan is one of the central questions in developmental biology (Arthur, 2000). A long studied subject, it seems established that evolutionary rates of different characters and lineages are different. Our results in Fig. 3 support Davidson and Erwin's theory that the hierarchical structure of the gene regulatory network has imposed constraints on the rate of further evolution of the most basic, and earliest-evolved features. The slow speed of evolution of the kernels that control the development of animal phylum- and superphylum-level body plan characteristics is why no new phylum-level body plans appeared after the pre-Cambrian period. The number of types of classes, orders, families, and species is increasing, and our results show that this observation is surprisingly consistent with the increasing evolutionary speed from kernels to plug-ins to I/Os to batteries. We propose that the slow evolution of the top components and fast evolution of the bottom components of the hierarchy is a universal phenomenon in evolution, not only in the gene regulatory networks, but also in protein interaction networks, cell signaling networks, and metabolic networks (Deem, 2007).

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